EXHIBIT 4

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Short reports

Trisomy 2q11.2→q21.1 resulting from an unbalanced insertion in two generations

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Abstract

In this communication, we describe two cases of proximal 2q trisomy (2q11.2→q21.1) resulting from an interchromosomal insertion. The chromosomal origin of the insertion was confirmed by fluorescence in situ hybridisation. An unbalanced karyotype, 46,XX,der(8),ins(8;2)(p21.3;q21.1q11.2), was found in the proband and her mother, who both have mild mental retardation, short stature, dysmorphic features, insulin dependent diabetes mellitus, and a psychotic illness. This family is a rare example of direct transmission of a partial autosomal trisomy.
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Keywords: chromosome 2; chromosome 8; insertion; trisomy

The inheritance of autosomal aneuploidy or partial monosomy/trisomy from a non-mosaic aneuploid parent is rare. The majority of cases reported involved direct transmission of small interstitial or terminal deletions.[1–11] Direct transmission of autosomal trisomies is exceptionally rare. There have been 10 cases described of trisomy 21 liveborns to trisomy 21 mothers.[12 13] In addition, direct transmission of duplications of 7p,[14] 8p,[15] 9p,[16] and 14q[17] have been reported.

In this paper we describe the identification of two cases of proximal 2q trisomy resulting from an interchromosomal insertion, confirmed by fluorescence in situ hybridisation. The unbalanced karyotype was found in both mother and daughter, representing a rare example of a partial autosomal trisomy in two generations.

Figure 1 Clinical photographs of the proband, (A) frontal and (B) lateral.

Case reports

PATIENT 1

The proband, a 37 year old female, first came to medical attention as a result of her unusual appearance and delay in language acquisition, her first words being recorded at 3 years of age. Subsequently, she received special education, but did not experience any medical problems until the diagnosis of insulin dependent diabetes mellitus (IDDM) was made at the age of 29 years. A diagnosis of a simple paranoid psychotic state was first made at the age of 31 years and this has been responsive to major tranquilliser treatment. The patient lives independently but works within a sheltered workshop and, although she has a wide vocabulary, writes only her name. Examination at 37 years showed that she is of short stature (152.5 cm, 5th centile) and moderately obese with mild microcephaly (OFC 52 cm, <3rd centile). Her secondary sexual characteristics were normal and her menarche was recorded at the age of 16 years. She has a brachycephalic skull and mild micrognathia, parted central incisors, a prominent columella, and low set ears with a very obvious crus helices (fig 1), but is otherwise normal in appearance.

PATIENT 2

The proband's mother was traced and found to be residing in a chronic psychiatric institution on long term psychotropic medication treatment as a result of her schizoaffective disorder (DSM IV 295.70). This illness manifested initially at 23 years with visual and auditory hallucinations and has also been characterised by paranoid delusional ideation and aggressive episodes. Her IQ was estimated to be 70 and she is able to function in a semi-independent manner, hospitalised largely because of her psychiatric state. She also has IDDM with the age of onset being recorded at 33 years and has a diabetic background retinopathy as a result. In addition, she receives thyroid hormone replacement and has a pelviureteric obstruction. At the age of 66 years, she is obese with mild short stature (154 cm, 10th centile) and a

Figure 2 Facial photograph of the proband's mother.

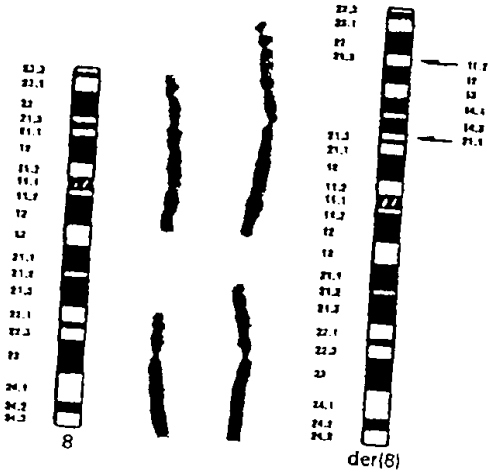

Figure 3 (A) Partial karyotype and (B) ideogram of the GTG banded chromosomes 2 and 8 from the proband.

normal OFC (55 cm). Her other body measurements were normal and she has a similar appearance to that of her daughter (fig 2). She had one other child (with a different partner) who had died of unknown causes aged 3 days. She herself was one of eight children with no known problems. Other family members were unavailable for study.

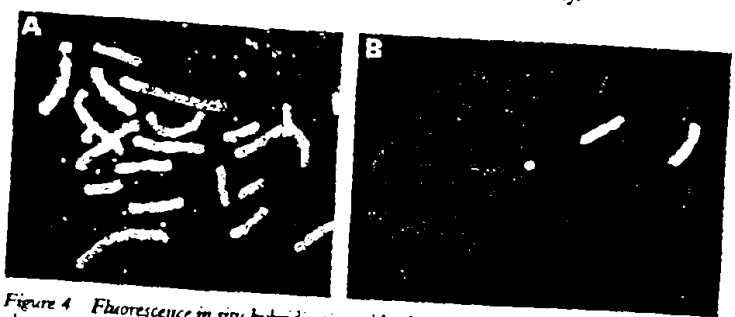

Figure 4 Fluorescence in situ hybridisation with whole chromosome paints for (A) chromosome 8, showing an insertion in the middle of the short arm of one of the chromosomes 8 and (B) chromosome 2, showing the insertion to be derived from chromosome 2.

CYTOGENETIC STUDIES

Cytogenetic analysis of cultured peripheral blood lymphocytes from the proband identified extra chromosomal material on the short arm of one of the chromosomes 8 (fig 3). Fluorescence in situ hybridisation (FISH) using a whole chromosome paint (wcp) for chromosome 8 (Cambio, Cambridge, UK) was performed according to the method of Pinkel *et al*[18] using avidin-fluorescein (Vector, Burlingame, CA) detection. FISH analysis showed that the extra material on the der(8) chromosome was an insertion in the middle of the short arm (fig 4A). Based on the GTG banding pattern of the inserted material, FISH analyses were performed with whole chromosome paints for the X chromosome (Cambio), chromosome 12 (Cambio), and chromosome 2 (Vysis, Downer's Grove, IL). The region of the insertion showed no signal with either the wcpX or wcp12 (data not shown). However, FISH with a wcp2 probe showed that the insertion was derived from chromosome 2 (fig 4B). Cytogenetic analysis of the proband's mother identified the same unbalanced karyotype (data not shown). The proband's father was dead, and other family members were unavailable for study. Based on the GTG banding pattern and FISH analyses the proband's karyotype was interpreted as an inverted insertion of 2q11.2→q21.1 into the short arm of chromosome 8: 46,XX,der(8),ins(8;2)(p21.3;q21.1q11.2)mat.ish der(8)(wcp2+,wcp8+).

Discussion

The two patients described here have trisomy for proximal 2q (2q11.2→q21.1), confirmed by fluorescence in situ hybridisation, resulting from an interchromosomal insertion. Common features that exist between the proband and her mother include mild mental retardation, short stature, a number of minor dysmorphic facial features, as well as insulin dependent diabetes mellitus (type I) and a psychotic disorder characterised by paranoid delusions.

Trisomy for proximal 2q is extremely rare. The only other report of a similar aneusomy was a patient with a smaller trisomy, 2q11.2→q14.2, resulting from a tandem duplication.[19] The common clinical features that appear to exist between the patients reported here and the case of Mu *et al*[19] include mental retardation, short stature, brachycephaly, and a prominent columella. However, the proband and her mother, in our case, had lesser mental handicap than the patient of Mu *et al*,[19] despite a larger trisomy. Neither of our patients had glaucoma, so it is possible that this was a coincidental finding in the patient of Mu *et al*.[19]

As shown by both the proband and her mother, proximal trisomy 2q does not appear to be severely debilitating. In fact, were it not for the investigation of the proband, the mother would not have been ascertained. No other family members were available for study. However, none was reputed to have had any problems that might suggest they had inherited the same imbalance. The origin of the der(8) in

the proband's mother could have been from malsegregation of a balanced parental insertion. Alternatively, given the mother's seven normal sibs, a de novo unbalanced insertion is also a possibility. The scarcity of patients with trisomies of proximal 2q is not surprising since malsegregation of a parental reciprocal translocation with a proximal 2q breakpoint resulting in 2q trisomy would inevitably be non-viable. Thus, tandem duplications and insertions are the most likely mechanisms through which such a trisomy would occur in a liveborn.

Interestingly, the index case and her mother both have insulin dependent diabetes mellitus (type I) and evidence of a major psychosis, with similar age of onset for both conditions. Neither of these disorders has been observed in the other case of trisomy reported for proximal 2q,[19] suggesting that either these effects were not expressed in this instance or that the causative genes are located between 2q14 and 2q21.2. It is possible that the presence of insulin dependent diabetes mellitus (type I) and a major psychosis in the proband and her mother are attributable to the disruption of genes in 8p or as a result of a position effect because of the insertion or both. To date, no assignment of gene(s) responsible for insulin dependent diabetes mellitus (type I) to 8p has been made. Genome screening for IDDM susceptibility genes indicated 2q34 as a likely candidate region for a diabetes susceptibility gene,[20] but this is distal to the breakpoints in our patients. However, more than one IDDM susceptibility gene on 2q may exist[20] so the intriguing possibility remains that increased dosage of a 2q IDDM susceptibility gene may be involved in causing diabetes in these people. Both mother and daughter have required major tranquilliser medication to control psychotic episodes characterised, in both instances, by paranoid features. Interestingly, schizophrenia has shown linkage to 8p in some studies,[21 22] but not others.[22] Aschauer *et al*[24] examined the 2q21 region for linkage to schizophrenia and schizophrenia related disorders but did not find evidence of linkage in the 14 schizophrenia families investigated so far.

Overall the phenotype seen in this familial insertion is surprisingly mild especially considering that the trisomic region of 2q involved is relatively large. This might suggest that either the function of genes expressed from this segment is not dosage dependent or that the effect of overexpression of genes from 2q is relatively benign. Several cases of aneuploidy associated with apparently normal phenotypes have been reported, indicating that increased dosage of genes in some regions of the genome may have little or no detrimental effect.[25-27] Bortotto *et al*[25] suggested that an imprinting effect might de facto correct any dosage imbalance resulting from the aneuploidy. However, Bernasconi *et al*[26] recently reported a phenotypically normal woman with maternal UPD2, indicating that imprinting is an unlikely mechanism to account for the mild phenotype in our patients.

In cases of parental aneuploidy, a 1:1 ratio of normal to aneuploid gametes would be expected, giving a theoretical recurrence risk of 50%. In reality, the observed recurrence risk appears to depend on the severity of the aneusomy. Rani *et al*[12] reviewed 31 pregnancies of trisomy 21 women with a incidence of normal 18:10 trisomy 21 liveborn offspring. The deviation from an expected 1:1 ratio of normal to affected offspring was almost certainly because of gestational loss. In contrast, there is unlikely to be any significant selection against smaller aneusomies. The majority of Charcot-Marie-Tooth type 1A disease patients and up to 25% of velocardiofacial patients are familial with no apparent deviation from a 1:1 ratio in offspring.[8 9 29] Since some aneusomies appear to be without phenotypic effect,[25-27] any selection will depend not only on the size, but also the genetic content, of the region.

As shown here and in previous reports of direct transmission of partial autosomal aneuploidy,[1-11 14-17] chromosomal imbalance for many regions can indeed be associated with fertility. Given the high recurrence risk (50%) involved in such cases, genetic counselling of such people and their guardians is strongly warranted.

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